Report

Percutaneous Absorption of Nicardipine and Ketorolac in Rhesus Monkeys

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Vehicle effects on the percutaneous absorption of nicardipine base, nicardipine hydrochloride, ketorolac acid, and ketorolac tromethamine were determined using the rhesus monkey as an in vivo model for human skin penetration. Vehicles investigated included blends of propylene glycol, trimethylene glycol, ethanol, Azone, Tween 20, water, and long-chain fatty acids. Formulations were prepared such that the compound dose, application area, and percentage saturation of the compound in the vehicle were held constant. Variations in absorption of the compounds were therefore attributable to vehicle effects. Each formulation was applied to three monkeys for a period of 24 hr using 10 Hill Top Chambers. Plasma samples were taken at appropriate intervals for 36 to 48 hr. The results indicated that trimethylene glycol and Tween 20 did not enhance absorption of the test compounds despite claims by other investigators. Azone and ethanol provided moderate enhancement of both the rate and the extent of absorption, while long-chain fatty acids in combination with propylene glycol significantly enhanced penetration. In general, higher fluxes were observed with the more lipophilic compounds nicardipine base and ketorolac acid as compared to the hydrochloride and tromethamine salts.

KEY WORDS: percutaneous absorption; nicardipine; ketorolac; penetration enhancers; vehicle effects.

INTRODUCTION

An analogy has often been made between the percutaneous absorption of drug compounds and solute diffusion through a membrane (1–6). The complexities of human skin are generally ignored and the stratum corneum is considered to be the rate-limiting membrane through which the solute must penetrate. Based on irreversible thermodynamics, the steady-state flux, or diffusion rate per unit area, of a solute through a membrane may be generally expressed as

\[ J = -L \frac{d}{dx} \left( \frac{u}{T} \right) = -L \frac{RT}{a} \frac{d}{dx} \left( \frac{a}{T} \right) \]  

where \( J \) is the flux, \( L \) is the Onsager phenomenological coefficient for diffusion, \( u \) is the chemical potential of the solute, \( T \) is the temperature, \( a \) is the thermodynamic activity of the solute, and \( R \) is the ideal gas constant (7,8). At a constant temperature, therefore, the driving force behind the diffusional process is the thermodynamic activity gradient across the membrane, \( da/dx \). Equation (1) simplifies to the well-known Fick’s first law of diffusion only when it is also assumed that all solutions are ideal:

\[ J = LR \frac{da}{dx} = -D \frac{dc}{dx} \]  

where \( c \) it the concentration of the solute and \( D \) is the diffusion coefficient of the solute. This assumption of ideality has often been ignored in discussions on membrane permeability, resulting in undue importance being placed on the concentration gradient.

The concept of activity gradient-driven diffusion becomes especially important when considering vehicle effects on percutaneous absorption. Since the thermodynamic activity of a solute is maximized at the solubility limit, the activity gradient and, therefore, the diffusional flux may be maximized by using a saturated donor phase. Absorption enhancement due to vehicle effects, therefore, becomes apparent when nonidentical fluxes are obtained with different vehicles at a constant thermodynamic activity (not a constant concentration) (9).

In this study, vehicle effects on the percutaneous absorption of drug compounds were determined using the rhesus monkey as a model for human skin penetration. Studies have indicated that the rhesus monkey is a good animal model for this purpose (10). Nicardipine (free base), nicardipine hydrochloride, ketorolac (free acid), and ketorolac tromethamine were selected as test drug compounds (see Fig. 1).

Several vehicle solvents were selected based on poten-
Fig. 1. Drug compounds used in this study: (A) nicardipine; (B) nicardipine hydrochloride; (C) ketorolac; (D) ketorolac tromethamine.

Potential absorption enhancement. In particular, propylene glycol has been reported to enhance percutaneous absorption in some cases (11). Trimethylene glycol has been claimed as a skin penetration enhancer for nifedipine and nicardipine (12). Ethanol has been shown to increase the skin permeation rate of estradiol (13). Cooper has published results indicating that blends of polar solvents, such as propylene glycol, and long-chain fatty acids, such as linoleic and oleic acids, increase the permeability of lipophilic compounds (14). Azone (1-dodecylazacycloheptan-2-one) is claimed to be a penetration enhancer and several investigators have reported skin penetration enhancement in the presence of surfactants (11,15–17). Vehicles composed of a combination of two or three of these solvents were chosen for use in this study.

MATERIALS AND METHODS

Materials. Nicardipine, nicardipine hydrochloride, ketorolac, and ketorolac tromethamine were provided by Syntex Corporation (Palo Alto, Calif.). Propylene glycol, USP (PG) (Syntex Corporation, Palo Alto, Calif.), trimethylene glycol (TMG) (1,3-propanediol; Eastman Kodak Co., Rochester, N.Y.), linoleic acid (LA) (Sigma Chemical Co., St. Louis, Mo.), oleic acid, NF-FCC (OA) (J. T. Baker Chemical Co., Phillipsburg, N.J.), Azone (AZ) (Nelson Research and Development, Irvine Calif.), Tween 20 (TW) (ICI Americas, Inc., Wilmington, Del.), and alcohol, USP (95% ethanol) (E) (190-proof alcohol, USP; U.S. Industrial Chemicals), were used as obtained. Twice deionized water (W) was used in some vehicles. Hill Top Chambers (HTCs) (18) were used as received from Hill Top Research.

Solubility Studies. Although the skin temperature of rhesus monkeys is higher, solubility studies were carried out at 25°C. In assuming maximum thermodynamic activity, therefore, the temperature coefficient of solubility for each compound was also assumed to be identical in each of the vehicles. The solubilities of nicardipine and nicardipine hydrochloride in PG/W, TMG/W, PG/LA, PG/W/AZ, PG/LA/W, and E/W were determined in triplicate by placing excess compound with each vehicle blend in a screw-capped vial. The vials were then rotated for 3 days in a 25°C water bath. Upon removal, the suspensions were filtered through 0.45-μm membrane filters, diluted in mobile phase, and assayed for drug content by high-performance liquid chromatography (HPLC). The solubilities of ketorolac and ketorolac tromethamine in PG/W, PG/LA, PG/OA, PG/W/TW, and E/W were also determined.

Percutaneous Absorption Studies. Formulations were chosen based on the results of the solubility studies. A 10-ml solution of each formulation was prepared, the appropriate volume of which was then loaded into each HTC using a 1000-μl glass syringe fitted with an 18-gauge needle.

For each formulation, three female rhesus monkeys (Macaca mulatta) weighing 6 to 9 kg were used. Chest hair was clipped closely rather than shaved in order to ensure that the stratum corneum remained intact. Prior to dosing, the animals were lightly anesthetized with 5 to 8 mg/kg ketamine. Each formulation was then applied over a total chest area of 27 cm² using 10 HTCs per monkey held in place by a single strip of adhesive tape. Three-milliliter blood samples were taken from the saphenous vein prior to dosing and at appropriate intervals over a period of 36 to 48 hr. The monkeys were restrained in metabolism chairs during the 24 hr application period. Following HTC removal, the application site was washed with soap and water. Plasma levels of compound were determined by HPLC (ketorolac and ketorolac tromethamine) (19) or a capillary column gas chromatographic (GC) method with electron capture detection (nicardipine and nicardipine hydrochloride) (20).

Compound Remaining Assay. Following removal of the HTCs from each monkey, the cotton swatches from 5 of the 10 HTCs were removed, placed in a 50-ml beaker with 30 ml of acetonitrile, methanol, or alcohol, U.S.P., and sonicated for 15 min. The extract was then filtered through a 0.45-μm membrane filter into a 100-ml volumetric flask. After two additional rinses, the extract solution was made to volume, diluted in mobile phase, and assayed for drug content by HPLC.

HPLC Analytical Methods. For nicardipine and nicardipine hydrochloride, the retention time was 7.8 min using 50:50 CH₃CN:0.05 M KH₂PO₄ at 1.0 ml/min through a Whatman Partisil 5 ODS-3 column. Detection was by UV absorption at 237 nm. For ketorolac and ketorolac tromethamine, the retention time was 6.4 min using 40:60:0.2 CH₃OH:CH₃OH:CH₃COOH at 1.3 ml/min through an ASI-C8 (10 μm) column. Detection was by UV absorption at 254 nm.

RESULTS AND DISCUSSION

Solubility Studies. Since the development of therapeutically effective formulations was the ultimate goal, an appropriate compound dose, d, was selected for each compound. Both the area of application and the allowable donor phase volume range, v, were fixed based on the use of 10 HTCs for formulation application. Therefore, in order to maintain a constant percentage saturation, f, in the vehicle, a solubility limit, s, falling within a specified range of values was required:

\[
0.286 \leq \frac{d}{v} \leq \frac{2.00 \times d}{f}
\]  
(3)